Effect of nutrient on growth of dwarf *Kandelia candel* (L.) Druce evaluated by grey relational analysis

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Abstract

Grey relational analysis was employed to study the effect of nitrogen (N) and/or phosphorus (P or NP) nutrients on the growth of dwarf *Kandelia candel* (L.) Druce in the Chuwei Mangrove Nature Preserve. A field fertilization experiment was designed using ammonium sulfate and superphosphate as the main sources of N and P. Shoots with at least 8 pairs of leaves, of which the two oldest pairs were yellowing, were collected at one year after fertilization. Chlorophyll content of eight proposed stages, including pigment synthesis and degradation, was determined. The primitive sequence data were constructed, and the grey initial normalization and mean normalization were calculated. For chlorophyll content and a/b ratio, the grey relational grade and order, based on both initial and mean normalization, is \( \Gamma_P > \Gamma_{NP} > \Gamma_N \), indicating the similarity with non-fertilizer plants is \( P > NP > N \). In other words, P treatment affected chlorophyll accumulation and the a/b ratio patterns were less than NP and N treatment. Thus, the order of the factor importance, which is the reverse sequence of the grey relational grade, is \( N > NP > P \). The grey relational analysis strongly indicated that N is the most important factor affecting the synthesis and degradation of chlorophyll in dwarf *K.*
營養因子對矮生帶水筆仔生長影響之灰關聯分析

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摘要

本研究利用灰關聯理論分析營養物質氮(N)和磷(P)對竹圍紅樹林自然保護區內矮生帶水筆仔(Kandelia candel (L.) Druce)的生長影響。田野施肥試驗利用硫酸銨和過磷酸鈣為植株氮和磷的來源，施肥一年後，收集第一對幼葉至第八對完全展開葉，其中包含黃化的兩對最老葉，測定八個包含色素合成和降解階段中的葉片葉緣素含量，構建原始序列數據及計算灰初值化或均值化。依初值化或均值化後的灰關聯運算，氮、磷對葉緣素含量和葉緣素 a/b 比值之灰關聯排序依序是 P>NP>N，此結果顯示與未施肥植株最相近者為 P，其次為 NP，再者為 N，亦即 P 處理最不影響葉緣素生合成及 a/b 比值之變化趨勢。因此，影響矮生帶水筆仔葉緣素含量的營養因子之順序為 N>NP>P。氮是影響矮生帶水筆仔葉緣素合成和降解的最重要因素，磷不如氮重要，但氮、磷的交互作用和氮具相同的影響效果。
Introduction

Mangrove is a group of trees or shrubs that grow in the tidal waters of tropical and subtropical coastlines. Factors such as high salinity, poor aeration (Davis, 1940), waterlogging (Egler, 1952), compacted peat (Craighead, 1971), nutrient-deficiency (Feller, 1995; Lugo and Snedaker, 1974), water availability, and salinity fluctuation (Lin and Sternberg, 1992a and b) have been thought to limit their growth.

*Kandelia candel* (L.), the only mangrove species in the Chuwei Mangrove Nature Preserve, on the coast of northwestern Taiwan, exhibits three distinct growth forms. The tall population is 4 m or greater; the dwarf population is about 1 m; and the medium population is 2~3 m. Long-term water use efficiency and stable isotope techniques have been used to study the factors responsible for the different growth forms of *K. candel* (L.) in the Chuwei Mangrove Nature Preserve. It was concluded that water and nutrient availability, especially nitrogen and phosphorus, limited the growth of *K. candel* (L.) (Kao and Chang, 1998). Hwang and Chen (1998) applied ammonium sulphate and superphosphate as sources of nitrogen and phosphate to the dwarf population within the preserve and concluded that nitrogen is the major limiting factor of the growth of *K. candel*.

Grey system theory was first appeared in 1982 (Deng, 1982 and 1989). The theory deals with the poor, incomplete, or uncertain problems of a system. The terms “white” and “black” mean that all the information in the system is completely known or unknown, respectively. The term “grey” means that part of information is known and some is incomplete or uncertain. Grey relation analysis (GRA) within grey system theory is meant to describe the posture relationship between one main factor and all the other factors in a given system. The known information is based on standard patterns (reference or control patterns). The grey relational grade thus is used to reflect the relationship between a reference condition and a test condition. Grey relational analysis has been used to find out the most important factor affecting a subject, such as a
topological effect on an ecological environment, serum marker’s effect on the detection of liver fibrosis, the relationship between main quality and agronomic characters of wheat, and so on (Che and He, 1993; Chen, 1993; Chen and Tan, 1995; Guo et al., 1991). Results based on grey relational analysis appeared more precise than those based on other analysis techniques under the same conditions.

In the present study, we applied ammonium sulfate and superphosphate to the dwarf population and used grey relational analysis to pinpoint the most important nutrient factor limiting the growth of dwarf *K. candel* in the Chuwei Mangrove Nature Preserve.

**Method and Materials**

*Experimental design.* Eight quadrates (5x5 m) in the dwarf population were chosen for fertilizer application of four 150 g doses of ammonium sulfate (21% available NH$_4^+$) or/and of superphosphate (16% water-soluble P$_2$O$_5$) as the N, P, and NP source. Fertilizer, enclosed in a dialysis tubing (15 cm long and 5 cm wide), was applied evenly at the center of each quadrant to a depth of 30 cm according to the method of Feller (1995). In a control quadrant, holes were cored and sealed without adding fertilizer. The leaves were collected at one year after fertilization.

*Leaves.* The leaves were collected and divided into eight groups according to their phyllotaxy, i.e. yellow green, light green, green, dark green, green, green-yellow, yellow-green and yellow—and designated, respectively, as stages 1~8.

*Chlorophyll determination.* Following extraction of liquid nitrogen-frozen leaf with 80% acetone, the concentrations of chlorophyll a and b and the ratio of chlorophyll a to b were determined according to the spectrophotometric method of Porra et al (1989). Absorbance was measured with a Hitachi U-2000 UV-visible spectrophotometer.

*Grey relational analysis.* The chlorophyll content or a/b ratio of N, P, NP used as a test series, and the chlorophyll content or a/b ratio of non-fertilizer control were used as a reference series. The basic principle and method of grey relational analysis are as follows (Deng, 1982 and 1989).

1. Normalization of original data series:
Either initial normalization or mean value normalization method is applied in the data series treatment. For example:

If the original data is \( x_0(k) = (x(1), x(2), x(3), \ldots, x(k)) \), then the initial normalization data series is

\[
\hat{x}_0 = \left( \frac{x(1)}{x(1)}, \frac{x(2)}{x(1)}, \frac{x(3)}{x(1)}, \ldots, \frac{x(k)}{x(1)} \right).
\]

If the mean value of the original data is \( \bar{X} \), then the mean value normalization data series becomes

\[
\hat{x}_0 = \left( \frac{x(1)}{\bar{X}}, \frac{x(2)}{\bar{X}}, \frac{x(3)}{\bar{X}}, \ldots, \frac{x(k)}{\bar{X}} \right),
\]

where \( \bar{X} = \frac{1}{n} \sum_{k=1}^{n} x(k). \)

(2) The grey relational coefficient \( \gamma \) is defined as follows:

\[
\gamma(x_0(k), x_j(k)) = \frac{\min_j \min_k \left( \left\| x_0(k) - x_j(k) \right\| + \zeta \max_j \max_k \left( \left\| x_0(k) - x_j(k) \right\| \right) \right) \left( \left\| x_0(k) - x_j(k) \right\| \right)}{\max_j \max_k \left( \left\| x_0(k) - x_j(k) \right\| \right) \left( \left\| x_0(k) - x_j(k) \right\| \right)}
\]

where

\[ j = 1, \ldots, m; \quad k = 1, \ldots, n \]

\( \zeta \) is identification coefficient, its value is taken \([0,1]\), usually,

\[ \zeta = 0.5. \]

\( x_0 \) is the reference data series.

\( x_j \) is the test data series.

\( \left\| x_0(k) - x_j(k) \right\| \) is the absolute value (norm) of the difference between \( x_0(k) \) and \( x_j(k) \).

\[ \min_j \min_k \left( \left\| x_0(k) - x_j(k) \right\| \right) \] is called the secondary minimum difference which is selected in all \( j \).

\[ \min_k \left( \left\| x_0(k) - x_j(k) \right\| \right) \] is called the first minimum difference which
is selected in all $k$.

all the same as $\max \max$.

(3) The grey relational grade \( \gamma^*(x_0(k), x_j(k)) \) is

\[
\gamma^*(x_0(k), x_j(k)) = \frac{1}{n} \sum_{k=1}^{n} \gamma(x_0(k), x_j(k)).
\]

(4) Rearrangement of grey relational grade from large value to little value in a series.

The grey relational grade has the following characteristics:

(1) \( 0 \leq \gamma^* \leq 1 \).

(2) The more similar the test data \( x_j(k) \) with the reference data \( x_0(k) \), the larger the grey relational grade value \( \gamma^* \).

(3) Only if the reference series \( x_0(k) \) is exactly equal the test series \( x_j(k) \), the grey relational grade value \( \gamma^* = 1 \).

**Results and Discussion**

Photosynthesis is initiated by the absorption of sunlight radiation energy by chlorophyll molecules. The chlorophyll quality and quantity are, therefore, key determinants of the plant’s photosynthetic efficiency. Chlorophyll accumulation, under any conditions, is the combined effect of chlorophyll synthesis and degradation (Matile, et al 1996; Reinbothe and Reinbothe, 1996). Under normal conditions, the chlorophyll synthesis of dwarf *K. candel* is slower than that of the tall population, whereas the chlorophyll degradation of the dwarf is faster (data not shown).

In this research, chlorophyll content and the a/b ratio were used as indicators of growth of *K. candel* dwarf population in Chuwei Mangrove Nature Preserve. The original data of these indicators are shown in Table 1. The chlorophyll content of the tall
population is greater than that of the dwarf at each corresponding stage. The chlorophyll accumulation and a/b ratio patterns of the two populations are similar. The chlorophyll contents in both tall and dwarf populations of *K. candel* increase as the leaf develops. They reach a peak at the fourth stage then decrease and finally disappear as the leaf senescences. During the same period, the chlorophyll a/b ratio of the tall population increases from about 3.2 to 3.9; the maximum is at the third stage; then decreases to about 1.5. The dwarf population, on the contrary, increases from 2.7 to 4.4, the maximum at the fourth stage, then decreases to about 1.0.

The supplementation of N- and NP-fertilizer obviously can restore the chlorophyll content and a/b ratio, but P-fertilizer alone can not. However, N supplementation seems unable to restore the chlorophyll content of dwarf *K. candel* back to the high level of the tall population (Table 1). The data apparently indicate that N, but not P, is the nutrient limiting factor affecting the growth of the dwarf population of *K. candel* in the Chuwei Mangrove Nature Preserve, reflected by the chlorophyll accumulation and a/b ratio. Although evaluating the influence of N and P on the leaf development and chlorophyll accumulation is easy, grey relational analysis is still used to confirm what the most important factor limiting the growth of dwarf population is. Both initial and mean normalizations of grey relational analysis were applied in this research.

The operation of grey relational analysis was described in Method and Materials. The chlorophyll content or a/b ratio of N, P, NP were used as a test series, and the chlorophyll content or a/b ratio of non-fertilizer control were used as a reference series. For chlorophyll content, while the grey relational grades (ΓN, ΓP and ΓNP) of N-, P- and NP-fertilized for dwarf *K. candel* (L.) Druce based on initial normalization were 0.625, 0.652 and 0.627, respectively, and based on the mean normalization were 0.646, 0.769 and 0.656, respectively (Table 2). For the chlorophyll a/b ratio, the grey relational grades for initial normalization were 0.545, 0.667 and 0.620 for ΓN, ΓP and ΓNP, respectively. Similarly, those grades for mean normalization were 0.522, 0.770 and 0.602, respectively. All of the above data showed the same grey order, that is P->N->NP- fertilizer. The results indicated the similarity with non-fertilizer plants is P > NP > N. In other words, P treatment affected chlorophyll accumulation and the a/b ratio patterns were less than NP and N treatment. Furthermore, the factor order of the importance, which is the reverse of the grey order and grey analysis grade, is
N-\rightarrow\text{NP-}\rightarrow\text{P-fertilizer}. Therefore, N is the most important factor affecting the chlorophyll accumulation and a/b ratio, whereas P is not as important as N; however, the combination of N and P have the same influence as N.

Table 1. Chlorophyll content and a/b ratio of dwarf *Kandelia candel* (L.) Druce leaf at proposed various development stages fertilized with a constant amount of N, P and NP for one year. Data are the mean of three determinations.

(A) Chlorophyll content (µg/g)

<table>
<thead>
<tr>
<th>Stages</th>
<th>Tall</th>
<th>Dwarf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>+N</td>
</tr>
<tr>
<td>1</td>
<td>611.3±28.9</td>
<td>420.4±32.7</td>
</tr>
<tr>
<td>2</td>
<td>877.1±22.6</td>
<td>598.3±19.4</td>
</tr>
<tr>
<td>3</td>
<td>1024.7±37.9</td>
<td>684.3±24.3</td>
</tr>
<tr>
<td>4</td>
<td>1413.3±47.1</td>
<td>812.6±11.4</td>
</tr>
<tr>
<td>5</td>
<td>841.9±35.2</td>
<td>428.3±25.1</td>
</tr>
<tr>
<td>6</td>
<td>378.5±18.8</td>
<td>214.2±16.3</td>
</tr>
<tr>
<td>7</td>
<td>100.2±3.6</td>
<td>58.2±10.1</td>
</tr>
<tr>
<td>8</td>
<td>58.0±3.3</td>
<td>24.9±4.7</td>
</tr>
</tbody>
</table>

(B) Chlorophyll a/b ratio

<table>
<thead>
<tr>
<th>Stages</th>
<th>Tall</th>
<th>Dwarf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>+N</td>
</tr>
<tr>
<td>1</td>
<td>3.18±0.18</td>
<td>2.69±0.15</td>
</tr>
<tr>
<td>2</td>
<td>3.95±0.24</td>
<td>4.15±0.22</td>
</tr>
<tr>
<td>3</td>
<td>3.94±0.27</td>
<td>4.31±0.16</td>
</tr>
<tr>
<td>4</td>
<td>3.75±0.14</td>
<td>4.38±0.13</td>
</tr>
<tr>
<td>5</td>
<td>2.30±0.15</td>
<td>3.08±0.11</td>
</tr>
<tr>
<td>6</td>
<td>1.83±0.19</td>
<td>2.38±0.11</td>
</tr>
<tr>
<td>7</td>
<td>1.61±0.13</td>
<td>1.28±0.06</td>
</tr>
<tr>
<td>8</td>
<td>1.49±0.17</td>
<td>1.02±0.05</td>
</tr>
</tbody>
</table>
Table 2. The grey relational grade and grey order of chlorophyll content and a/b ratio in dwarf *Kandelia candel* (L.) Druce leaf at proposed various development stages fertilized with a constant amount of N, P and NP.

(A) chlorophyll content

<table>
<thead>
<tr>
<th>Grey relational analysis</th>
<th>Initial normalization</th>
<th>Mean normalization</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$\Gamma_N$</td>
<td>$\Gamma_P$</td>
</tr>
<tr>
<td>grey relational grade</td>
<td>0.625</td>
<td>0.652</td>
</tr>
<tr>
<td>grey order</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>factor importance</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

(B) chlorophyll a/b ratio

<table>
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<td>1</td>
</tr>
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<td>factor importance</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Many characteristics of *K. candel* can be used as indicators of growth. Hwang and Chen (1998) used leaf area per shoot, new subshoots per shoot, first shoot biomass, leaf biomass per shoot and the N and P content of leaf tissue as the growth parameter to evaluate the relationship between N, P and NP and the dwarf population. Chlorophyll content is much easier to measure in a relatively short time and makes the analysis of the relationship between fertilizer-supplementation and the growth of *K. candel* possible.

The raw data apparently show that N, not P, is the key factor limiting the growth of *K. candel*. The result of the complex calculation of grey relational analysis further confirms the above relationship, regardless of the chlorophyll content and a/b ratio.
Literature Cited


Lin G, LSL Sternberg (1992b) Effect of growth form, salinity, nutrient and sulfide on photosynthesis, carbon isotope discrimination and growth of red mangrove